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Master in Quality of Food of Animal Origin

Effect of the level of concentrate in the diet of dairy sheep on the milk yield and cheese-yielding features of the milk

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Bellaterra, July of 2016

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DECLARATION

Prof. Dr. Gerado Caja and Prof. Dr. Jordi Saldo

INFORM that

The research work entitled "Effect of the level of concentrate in the diet of dairy ewes on the milk yield and cheese-yielding features of the milk" has been done under our supervision or guardianship by Mr. Lý Đắc Triệu Minh within the module Master's Thesis of the Master of Quality of Food of Animal Origin of the Autonomous University of Barcelona.

Bellaterra, 06 July of 2016

Dr. Gerado Caja　　　　　　　　　　　　　　Dr. Jordi Saldo

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ABSTRACT

Effect of the level of concentrate in the diet of dairy sheep on the cheese-yielding features of their milk

Diet is an important factor to contribute the final quality of dairy products in ruminants, in which the supplementation with concentrate is used to increase and to equilibrate the intake of nutrients, expecting to produce a higher milk yield or to modify its composition. Nevertheless, the effects of forage:concentrate ratio on milk composition and cheese-yielding performances are scarcely known in the case of dairy sheep. With this aim, an experiment was conducted using a total of 72 dairy ewes at early lactation and lasting 3 mo. Ewes of 2 breeds (Manchega, n = 36; Lacaune, n = 36) at d 60 of lactation were allocated in balanced groups of 6 ewes by breed. Experimental treatments consisted of different levels of concentrate (%, dry matter basis) added to a total mixed ration made of a similar forage and fed *ad libitum*; they were: Control (C, 30%), Medium (T1, 45%) and High (T2, 60%). Ewes were machine milked twice daily and milk yield automatically collected at each milking by using electronic identification and direct flow milk meters. Individual milk samples were collected on d 90 and 120 of lactation for composition and to evaluate the coagulation and cheese-yielding features of the milk according to treatments. Despite clear differences observed by ewe breed and stage of lactation, no differences were detected by the forage:concentrate ratio for all the milk yield and composition traits studied as well as on the cheese-yielding performances. In conclusion, under the feeding and management conditions used, the high- (Lacaune) and medium-yielding (Manchega) dairy ewes used may be fed with a wide range of concentrate (30 to 60%, on dry matter basis) without deleterious effects on milk and cheese-yielding traits. The optimal diet to be used in dairy ewes should depend on the forages and concentrate market prices and not on dairy performances.

RESUMEN

Efecto del nivel de concentrado en la ración de ovejas lecheras sobre la producción y características queseras de la leche

La alimentación es un importante factor determinante de la calidad final de los productos lácteos en rumiantes, en los que el suplementario con concentrado se usa para aumentar o equilibrar la ingestión de nutrientes, esperando producir así una mayor producción de leche o modificar su composición. Sin embargo, los efectos de la relación forraje:concentrado en la producción de leche y rendimiento quesero en ovejas lecheras es poco conocido. Con este objetivo, se llevó a cabo un experimento utilizando un total 72 ovejas lecheras a inicio de lactación y durante 3 meses. Ovejas de 2 razas (Manchega, n = 36; Lacaune, n = 36) a los 60 d de lactación se distribuyeron en grupos equilibrados de 6 ovejas por raza. Los tratamientos experimentales consistieron en distintos niveles de concentrado (%, sobre material seca) añadidos a una ración base de realizada con el mismo forraje y ofrecida *ad libitum*; estos fueron: Control (C, 30%), Medio (T1, 45%) y Alto (T2, 60%). Las ovejas se ordeñaron a máquina dos veces al día y su producción de leche se midió automáticamente en cada ordeño utilizando identificación electrónica y medidores de leche de flujo directo. A los 90 y 120 d de lactación, se recogieron muestras individuales de leche para analizar la composición y para evaluarlas propiedades de coagulación y rendimiento quesero de la leche según los tratamientos. A pesar de las claras diferencias observadas según la raza de ovejas y el estado de lactación, no se detectaron diferencias debidas a la relación forraje:concentrado en todas las variables de producción y composición de leche estudiadas, así como tampoco en las del rendimiento quesero. En conclusión, en las condición es de alimentación y manejo utilizadas, ovejas lecheras de alto (Lacaune) y medio (Manchega) nivel de producción pueden ser alimentadas con un amplio rango de concentrado (30 a 60%, sobre materia seca) sin apreciables efectos negativos en los caracteres lecheros y queseros. La ración optima a ser usada en ovejas lecheras, dependerá de los precios de mercado de los forrajes y concentrados y no de sus efectos en los rendimientos lecheros.

INTRODUCTION

1.1. Dairy sheep's milk at cheese-making industry

For thousands of year, sheep have been milked historically before any other animal. There were a lot of countries highly developed and common in dairy sheep industry, especially in and around the Mediterranean Sea with a common Greek-Roman culture. Sheep milk is superior for cheese-making to the milk from goats and cows because it is much richer in total solids, containing especially higher amounts of fat and protein (Table 1). For this reason, sheep milk gives a much higher cheese yield than the milk from cows or goats, beside that yogurt and ice cream are also common to be made from sheep milk.

Table 1. Average main composition of sheep, goat and cow's milk (Park *et al.* 2007)

	Milk component (%)				
Specie	Total solids	Fat	Protein	Casein	Lactose
Sheep	19.9	7.9	6.2	4.2	4.9
Goat	12.7	3.8	3.4	2.4	4.1
Cow	12.6	3.6	3.2	2.6	4.7

For improving the cheese yield and quality, it is necessary to evaluation the relationship between nutrition applied for dairy sheep and milk quality, which is highly depend on its technological and coagulation properties. These technological and properties are highly affected by milk fat and protein concentration and on the somatic cell content (SCC) which was not studied in this experiment. The contribution of milk protein is more significant than fat milk content, which was made clear in the study of several types of cheese (Table 2).

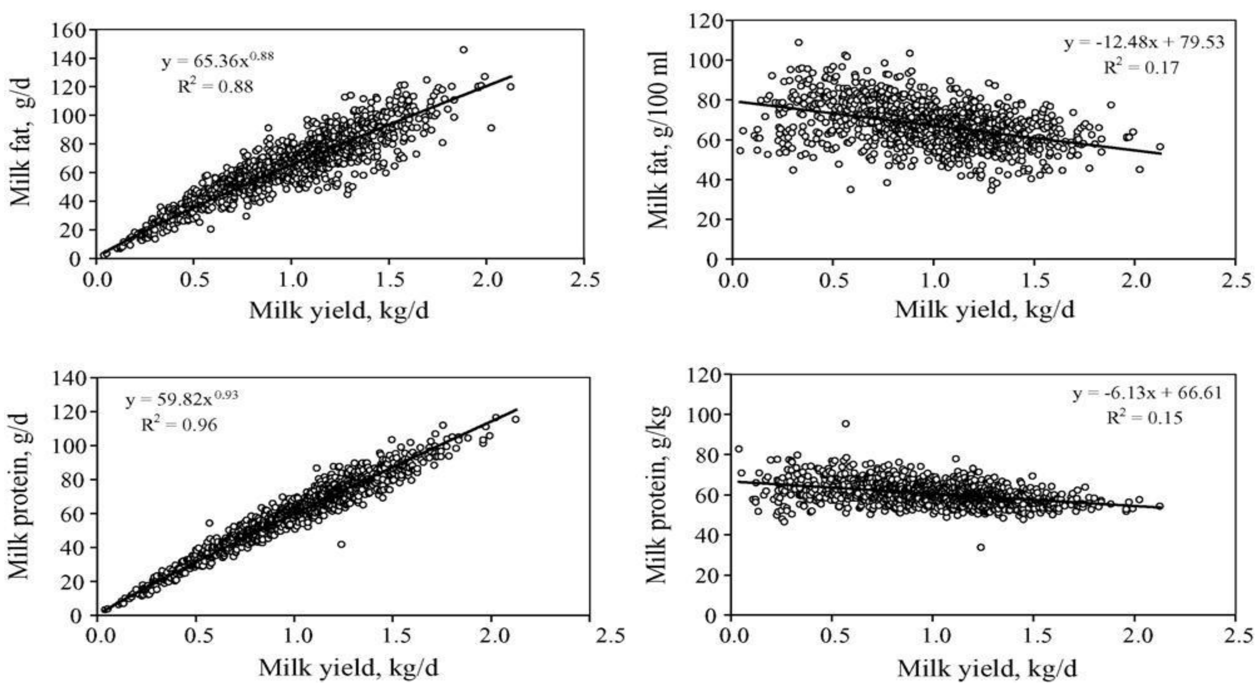
Table 2. Equations estimating cheese yield (Y, g cheese/100 g milk) as a function of fat (F) and protein (P) concentration in ewe's milk (Pulina *et al.* 2006).

Equation	R^2	Cheese type
Y = 1.747 P (g/100 mL) + 1.272 F (g/100 mL)	0.93	Pecorino Romano
Y = 1.733 P (g/100 mL) + 1.257 F (g/100 mL)	0.95	Pecorino Sardo
Y = 0.32 P (g/L) + 0.06 F (g/L) + 1.81	0.99	Roquefort

Therefore, the modification of protein and fat should be considered as the main goal and also the principle target to improve the sheep milk quality to produce the higher cheese yield and quality. There are a huge number of factors, including milk yield, genetic, phenotype and some other environmental factors with the different level of effectiveness that can affect the milk composition of dairy ewe and also the other dairy ruminants. Among them, milk yield was demonstrated to be the major factor influencing the milk fat and protein concentration. In sheep as similar with the other dairy ruminants, phenotypic and genetic correlations between milk yield and fat and protein concentrations are negative (Emery *et al.* 1988) and genetic correlations between milk yield and fat and protein concentrations are higher than those of environmental factors (Pulina *et al.* 2006). The reduction of fat and protein content of milk, as milk yield increases is well-known (Emery, 1988). Usually, as milk yield increases, the amount of lactose synthesized and secreted also increases, fat and protein synthesis generally increases at a slower rate.

Thus, the higher the milk yield, the more cheese produced per ewe, even though each additional unit of production results in a lower increase in cheese yield per liter. The variability is higher for fat yield when compared to protein yield, with the total variance accounted for by the model being 82–92% for fat and 92–98% for protein (e.g. Figure 1 for the Sarda breed). Because the relationship between milk fat concentration and yield has a higher variability than that between milk protein concentration and yield (Figure 1), modification of milk composition by nutritional means should be easier to achieve for fat than for protein.

Figure 1. Relationships between milk yield and components yield and content in 1,665 individual data of Sarda ewes (Pulina *et al*. 2006)



1.2. Effects of nutrition on the composition of sheep's milk

Type of breed, stage of lactation, milking system and feeding are important factors that can modify the sheep's lactation curves, in yield and milk composition (Treacher, 1983, 1989; Bocquier and Caja, 1993) similarly in other species of ruminants (Flamant and Morand-Fehr, 1982). Besides that milk yield and milk composition (fat, protein, casein and serum proteins, but not lactose) are negatively correlated in sheep and the other dairy species (Molina and Gallego, 1994; Fuertes *et al.* 1998). Level of nutrition, usually referred to energy level or feed intake level, is a main factor affecting milk yield and milk composition in dairy ruminants (Bocquier and Caja, 2000). It is generally accepted that there is a positive relationship between milk yield and level of nutrition. As a consequence, when we are improving the level of nutrition by applying the diet to increase the milk yield, we should pay attention to the correlated reduction of milk composition, so the optimized diet with the acceptable balance of milk yield and components is necessarily needed.

Moreover, as this document has mentioned above, the 2 important parameters to predict the cheese yield and quality of sheep milk is milk fat and protein contents. The significantly negative correlation of high level of nutrition with milk fat content and slightly positive correlation of this nutrition with milk protein and casein contents in were recorded. So, there were number of methods to improve the diet of increasing the protein or fat or concentrate intake, but the effects were not similar. The 3 common methodologies to improve nutrient intake are increase the protein, fat and concentrate intake. While, the increase in dietary protein concentration in the diet has no effect on milk fat or milk protein contents, feeding with concentrate (energy intake improving) may excess the rumen acidosis digestion leading to the depression of the milk fat and protein contents (Caja and Bocquier, 2000). In the other hand, it is reported that the possibilities of altering milk composition by feeding are higher for fat than for protein and/or casein contents (Sutton and Morant, 1989). Besides that, feeding strategy at lactation stage of dairy ewes is a principle factor to make the modification of milk yield and composition, so that every purpose of improving nutrient intake should be reasonably applying in that period. Hence, it has been accepted for a long time that improving nutrient intake at the middle and final stages of lactation has more effect than at the early of lactation (Bocquier and Caja, 1993). There are some reasons to explain that phenomenon, but it is mainly that with the increase of voluntary intake after lambing (Caja *et al.* 1997) when the good quality forages are fed *ad libitum*, the energy balance reaches the equilibrium within a few weeks after weaning in dairy sheep, the changes of milk composition and fat profile by different diets is expectedly expressed (Caja, 1994; Bocquier *et al.* 1995). Available references on the effects of different levels of nutrition in lactating ewes are summarized in Table 3.

Table 3. Ranges and sense of variation of milk yield and composition induced by the level of nutrition in lactating ewes (Caja and Bocquier, 2000).

Lactation period and reference	Sheep breed	Feeding level		Milk		
		Energy (UFL/g)	Protein (g PDI/d)	Yield (L/d)	Fat (g/L)	Protein (g/L)
Suckling						
Robinson *et al*. 1974	Cheviot	2.14-2.27	188-265	2.4-3.1	76-74	54-50
Cowan *et al*. 1980	F×D[1]	1.78-2.77	214-317	2.2-3.3	83-74	55-52
Cowan *et al*. 1981	F×D	2.28-2.33	241-277	3.3-3.5	84-92	53-56
González *et al*. 1984	F×D	1.66-2.36	183-260	2.3-2.6	90	50-52
González *et al*. 1984	F×D	1.66-2.36	212-302	2.3-2.7	90	52-54
González *et al*. 1984	F×D	1.66-2.36	239-339	2.5-3.1	90	53-54
Geenty and Sykes, 1986	Dorset	1.99-2.00	146	2.4-2.5	76	40-39
Geenty and Sykes, 1986	Dorset	1.51-2.42	138-170	2.0-2.7	79-69	40-39
Pérez-Oguez*et al*. 1994	Manchega	1.36-1.49	143-162	1.4-1.5	88-84	49
Milking						
Treacher, 1971	Dorset	1.06-2.18	107-221	1.2-1.5	83-68	46-52
Bocquier *et al*. 1985	F×S×L[2]	0.87-0.95	113-122	1.0	35-52	32
Geenty and Sykes, 1986	Dorset	1.83	124	1.7	71	47
Geenty and Sykes, 1986	Dorset	1.69-2.10	132-158	1.5-2.0	71-65	53
Pérez-Oguez *et al*. 1994	Manchega	1.41-1.50	147-164	0.6	92-99	57-58

[1]F×D = Finnish landrace × Dorset horn; [2]F×S×L = Finnish × Sarda × Lacaune.

As we can see the results in this table, were main results on dairy sheep nutrition are summarized, there are not clear correlations between energy and protein feeding strategies with the main characteristic of sheep's milk indifferent sheep breeds.

1.3. Effects of the level or proportion of concentrate in the diet on ewe's milk composition

As we have known there is a negative correlation between milk yield and fat and protein content of milk. Although the response of several breeds to concentrates are still different, in the paper of Bocquier and Caja published in 1993 they collected several available references of suckling and milking ewes in a wide range of energy balance (–1.5 to +1.5 UFL/d) and milk yield (0.6 to 3.5 L/d) conditions to confirm that milk fat content is negatively correlated (r = –0.87) to ewe's energy balance (–1 UFL/d = +12.2 g/L milk fat). Moreover, using a high proportion of concentrate in the diet of dairy ewes (higher than 50% of dry matter) is possibly leading to some negative effect for the both the milk fat and protein contents during the first months of lactation (Eyal *et al.* 1978).

An excess of concentrate in the diet can result in a quick physical-chemical degradation of non-structural carbohydrates (i.e., starch and sugars) in the rumen, which has some effects in the dairy sheep metabolism. Main effects are reducing dramatically the rumen pH and altering the amount and composition of microbial protein synthesis and also limiting the degradation of structural carbohydrates (i.e., cellulose) in the rumen (Hadjipanayiotou *et al.* 1988). Additionally, there is an increase of body weight and an improvement of body condition score in the case of lactating ewes fed by group with high levels of concentrate. Nevertheless, in dairy ewes, there are still lacking studies and knowledge about the effects of the forage and concentrate ratio (F:C) of the diet on milk composition, cheese-yielding features and fatty acids (FA) profile of the milk.

There are also several papers studying the responses of different species of ruminants to a variety of diets and lipid supplements. These responses of ruminant species are significantly different with regard to milk yield, milk components (fat and protein contents) and FA profile (Chilliard *et al.* 2003; Pulina *et al.* 2006, Sanz-Sampelayo *et al.* 2007; Tsiplakou and Zervas, 2008). In the specific case of dairy cows, feeding with high concentrate diets and vegetal-oils often decreases milk fat production (Doreau and Demeyer, 1999). In addition, there was recorded an increase of rumenic acid concentration (RA; *cis*-9, *trans*-11 C18:2), a conjugated linoleic acid (CLA) isomer with advantageous physiological effects (Lock *et al.* 2009). Similarly, the effects of the presence or absence of soybean oil supplementation in the diets of dairy ewes were studied by Antongiovanni *et al.* 2004 and Mele *et al.* 2006 using a F:C ratios of 75:25 or 60:40, respectively. The greater increase in RA and its precursor the vaccenic acid (VA; *trans*-11 C18:1) were obtained with the inclusion of soybean oil in the 75:25 ration, whereas the greater increase of *trans*-10 C18:1 level was obtained with the 60:40 ration. Moreover, data from more recent works also suggest that increases in *trans*-10 C18:1 content, when sunflower oil is supplemented into the diet of dairy ewes, could be related to the F:C ratio (Toral *et al.* 2010).

Although similar studies in dairy sheep may produce different results in comparison with those obtained in dairy cows, there is generally accepted that a higher proportion of concentrate in diet will induce changes in both milk composition (milk fat concentration is faster and easily changed than milk protein concentration) and FA profile (usually more saturated than unsaturated fatty acid).

1.4. Objectives

The main objective of this thesis of Master of Quality of Food of Animal Origin was to study the response on milk production and cheese-yielding performances of dairy ewes fed with different diets during mid-lactation. To study about this objective, an experiment was designed to analyze the values of the following variables:

- Milk yield
- Milk composition
  - Total solids
  - Fat
  - Protein
- Cheese-yielding performances:
  - Rennet coagulation time
  - Rate of curd aggregate
  - Firmness
  - Laboratory cheese yield

The experimental design included two different breeds of dairy ewes: Manchega and Lacaune, known by their similar body weight and different milk yield and composition.

MATERIALS AND METHODS

Animal care conditions and management practices undertaken in this project were approved by the Ethical Committee of Animal and Human Experimentation of the Autonomous University of Barcelona (Bellaterra, Spain; CEEAH reference 14/2807) and the codes of the Ministry of Agriculture, Food and Environment of Spain (Madrid). This experimental project was a collaborative work with the cooperation of El Hadi (PhD student of Faculty of Food and Animal Science) to get involved in milk composition analyses.

2.1. Experimental procedures

2.1.1 Animals and Management

The Manchega and Lacaune dairy ewes used in this experiment belong to the experimental flock of the SGCE (Servei de Granges i Camps Experimentals) at the Veterinary Faculty of the University Autonoma of Barcelona in Bellaterra (Barcelona). Both breeds were adapted for few generations (approximately 30yr) to the semi-intensive experimental farm conditions of the SGCE. The ewes lambed in autumn and then suckled their lambs which were weaned at 28-30 d of age. Ewes were machine milked twice-daily after the weaning of the lambs. A total of 72 healthy dairy ewes were selected from the 120 ewes of the flock and allocated into 6 balanced groups of 6 ewes each by breed, according to milk yield and body weight, to which the experimental treatments were randomly applied. All of the ewes wear conventional and electronic identification (i.e., plastic ear tags and electronic boluses) to be individualized and to record automatically their milk yield at each milking.

Treatments consisted of different total mixed rations (TMR) differing in the forage to concentrate ratio (on DM basis) and were: 1) Control (CO, 70:30%), 2) Treatment 1(T1, 55:45%) and 3) Treatment 2 (T2, 40:60%).

All the animals were maintained under indoors conditions, the temperature of the shelter ranging from 6.5 to 22.5ºC and the relative humidity ranging from 12 to 95%, and adapted to the experimental conditions using the same CO diet during a pre-experimental period of 30 d, from d 30 to 60 of lactation. Treatments were applied from 60 to 120 d of lactation.

The thermo hygrometric index (THI) is a simple index based on the maximum daily temperature and relative humidity in predicting the health effects of specific meteorological conditions, was calculated according to the NRC (1971) as (T = dry bulb ambient temperature, ºC); H = relative humidity, %) (cited by Dikmen and Hansen, 2009):

$$\mathrm{THI} = (1.8\ \mathrm{T} + 32) - (0.55 - 0.0055\ \mathrm{H}) \cdot (1.8\ \mathrm{T} - 26.8)$$

Observed THI values were ranged between 44 to 56, indicating moderately cold temperatures during the full experimental period and adequate for milk production in dairy sheep.

Milk performances (i.e., yield and composition) of all before experimental groups were stabilized applying the experimental diets at d 60 of lactation which was the starting day of the experiment. The allocation of diets and ewe's group characteristics are shown in Table 4.

Table 4. Allocation of experimental ewes by group and dietary treatment

Group	Replica	Breed	Diet	Ewes	Yield, L/d[1]
1	1	Manchega	Control (CO)	6	0.6
	2			6	0.6
2	3	Lacaune		6	1
	4			6	1
3	5	Manchega	Medium (T1)	6	0.6
	6			6	0.6
4	7	Lacaune		6	1
	8			6	1
5	9	Manchega	High (T2)	6	0.6
	10			6	0.6
6	11	Lacaune		6	1
	12			6	1

[1]Standard milk calculated according to Bocquier *et al*. 1985 and Pérez-Oguez *et al*. 1994.

Ewes were milked twice-daily (07:30 and 17:00h) using a double-12 stall parallel milking parlor (Amarre Azul I, DeLaval Equipos, Alcobendas, Madrid, Spain) with a central high milk pipeline, 12 DeLaval SG-TF100 milking clusters, and 12 MM25SG milk flow and recording meters (both from DeLaval, Tumba, Sweden). Milking was performed at a vacuum of 40 kPa, 120 pulses/min, and 50% pulsation ratio. The milking routine did not include teat preparation. The clusters were attached manually for machine milking, removed automatically when the milk flow rate was lower than 0.1 L/min or the milking time was higher than 3 min and finally, after cluster removal, the teats were dipped in a iodine solution (P3-io shield; Ecolab Hispano-Portuguesa, Barcelona, Spain) after milking.

The total milk amount of each single ewe was recorded daily, including the morning and evening milking yields through the experimental period. Data were uploaded automatically into the AlPro software (DeLaval, Tumba, Sweden) and weekly reviewed and saved in a previously designed Excel spread sheet.

2.1.2. Experimental diets

The aim of the 3 diets applied in this experiment was to increase the daily nutrient intake of the ewes during mid-lactation in order to assess on their mid-term effects on milk yield, milk composition and cheese-yielding features. We hypothesized that the range of increase of concentrate between treatments (15% of DM) will significantly modify the results of the measured variables.

Table 5. Feed ingredients offered daily to the ewes according to the experimental treatments

Ingredients	Control	Medium (T1)	High(T2)
Forage, kg/d (as fed)[1]	2.0	2.0	2.0
Concentrate, kg/d (as fed)[2]	0.3	0.3	0.3
Whole grain corn, kg/d (as fed)	0.5	0.5	0.5
Extra cornfed at milking, kg/d (as fed)[3]	0.2	0.4	0.6
Concentrate (%, DM basis)	30	45	60

[1]Alfalfa hay; [2]Composition: Corn 4%, Barley 10%, Oats 10%, Gluten feed 10%, Rapeseed meal “00” 5%, Soybean hulls 50%, Soybean oil 5%, Di-calciumphosphate (18%) 2.5%, Cane molasses 2%, Salt 0.5%, Vitamin premix 1% (VitafacOvino 0.3% (DSM Nutritional Products Europe, Switzerland), Vitamin A 3,333,333 IU/kg, Vitamin D3 3,333,333IU/kg, Vitamin E 5,666 mg/kg, Vitamin B1 666 mg/k, Vitamin B3 333 mg/kg, $FeCO_3$ 11,666 mg/kg, MnO 13,333 mg/kg, $3Co(OH)2.H_2O$ 66 mg/kg, ZnO 13,333 mg/kg, $Ca(IO_3)_2$ 166 mg/kg, Na_2SeO_3 100 mg/kg);[3]Distributed in 2 portions/d.

All of the feed ingredients used (forage and concentrate) were purchased from local suppliers and mixed in the experimental farm every 2 d by using a vertical mixer (TalleresCompar 8 m^3, Sant Pere de Torelló, Barcelona, Spain). Diet was weighed and distributed *ad libitum* 2 times per day (10:00 and 15:00h) and the orts removed and weighed, before providing the new diet of the next day. Daily feed intake was calculated by difference. Table 5 shows the composition of the diets. The extra corn added to the basal diet for the C, T1 and T2 treatments, was provided 2 times daily in the milking parlor manger at the start of milking, and the ewes consumed it during the milking process.

2.2. Sampling procedures

Milk samples for composition and cheese yielding were obtained individually using DeLaval milk samplers. The samplers took a representative volume of approximately 10% of the milk produced at each milking that were composited in a proportion of 60:40 with regard to AM:PM milking, according the respective milking intervals. Milk samples were collected from each ewe 3 times throughout the experiment, corresponding to the pre-experimental period (d 60), mid- (d 90) and late- (d 120) lactation periods (dates: November 18 and December 22 of 2015, and January 22 of 2016).

The cheese-yielding milk samples were collected to get the volume of 30 mL for each ewe at d 90 and 120. All of the samples were a similarly composited with the AM and PM milking samples (60:40, respectively). After collection, the samples were preserved with an antimicrobial tablet (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems, San Ramon, CA, USA) and stored in the refrigerator at approximately 4ºC for 24 h until analysis.

2.3. Experimental Analyses

2.3.1. Milk composition

Milk samples were conditioned to get a temperature of approximately 40°C before being analyzed by using a near infrared spectrometer (Foss Electric, Nordersted, Germany) for total solids (TS), fat, crude protein (N × 6.38), true protein, casein (CN) and lactose contents, as indicated by Albanell *et al.* 1999. Calibrations were performed using data obtained by conventional methods, including Gerber method for fat, Kjeldahl for nitrogen and oven at 103ºC for total solids (dry matter).

Energy corrected milk (ECM) standardized the amount of energy in the milk to 1.2 Mcal/L based upon fat and protein contents, according to the equation (Bocquier *et al.* 1993):

$$\text{ECM} = \text{Milk (L/d)} \cdot [0.071 \cdot \text{Fat (\%)} + 0.043 \cdot \text{Protein (\%)} + 0.22]$$

2.3.2. Cheese-yielding performance

Rennet coagulation solution: Diluted rennet (1:10) was prepared by mixing 500 μL of calf rennet with 4.5 mL of distillated water to create the 33 μL of 10-fold diluted rennet (calf origin, 1:1000 strength) for each 10 mL of testing sample.

Cheese-yielding evaluation: Three cheese-yielding traits (named RCT, RCA and F45) were determined in each milk sample by using the optical coagulation instrument (Optigraph, Ysebaert, Frépillon, France). Sets of 10 samples of 10 mL each, chosen at random, were tested simultaneously. Firstly, these samples were conditioned at the coagulation temperature of 33°C for 10 min. Thereafter 33 μL of the rennet coagulation solution, previously prepared, were added to each sample. Coagulation recording took place for approximately 45 min, getting 4 variables: time, optical density measure, 1stderivative and 2ndderivative. The RCT (rennet coagulation time) was determined by the time that the sample needed to get the maximum value of 1stderivative. This maximum value is also defined as RCA (rate of curd aggregation). The F45 (Firmness at 45 min) was determined by the result of the optical density value at min 45 less the value of the optical density at the time at when all the samples were stabilized.

Laboratory cheese yield (LCY): The test was based on the Othmane *et al.* 2002 methodology with some modifications using a Hettich Universal 32R (DJB Labcare, Buckinghamshire, England) centrifuge. All samples were conditioned to 33ºC before the addition of 33 μL of rennet solution. The coagulation was also done at 33ºC for 45 min, after that, obtained gels were vertically cut in across with a spatula. Therefore, they were centrifuged at 2500*g* for 15 min at 20ºC and the whey drained. The sample weights, before and after draining the whey, were recorded and used to calculate the LCY rate:

$$\text{LCY} = \frac{\text{Remaining solid weigh}}{\text{Total 10 mL milk sample weigh}}$$

2.4. Statistical Analyses

A descriptive statistical analysis was applied to all the response variables in order to know if there were some explanatory effects. All variables were normally distributed and the assumption of homogeneity of the variances was also checked using the Statistica Program (v. 7.0, StatSoftInc, Tulsa, USA). Explanatory and possible interactions were tested at first.

Data were analyzed by the PROC MIXED for repeated measurements of SAS (v. 9.1.3, SAS Institute Inc., Cary, NC, USA). The statistical mixed model contained the fixed effects of the treatment (control, treatment 1 or treatment 2) and the breed (Manchega or Lacaune), the random effect of the animal within breed (36 of each breed), and the standard error. Dependent variables analyzed were milk production (milk yield and milk composition) and cheese-yielding performances (rennet coagulation time, rate of curd aggregation, firmness at min 45, laboratory cheese yield). The model was:

$$Y_{ijkl} = \mu + T_i + B_j + A_k + \varepsilon_{ijkl}$$

Y_{ijkl}:dependent variable

μ : overall mean

T_i: treatment effect (i = Control, Medium and High)

B_j : breed effect (j = Manchega and Lacaune)

A_k : individual animal's random effect (k = 1 to 36)

ε_{ijkl} : residual error effect

Significance was declared at $P< 0.001$, $P< 0.01$ and $P< 0.05$, and tendency at $P< 0.10$.

RESULTS AND DISCUSSION

3.1. Milk yield

As we can see in Table 6 and Figure2, there were similar average milk yields for the 3 experimental groups of ewes at the start of the experiment (d 60), being on average 1.68 and 2.31 L/d for the Manchega and Lacaune breeds, respectively. Lacaune ewes produced greater quantity of milk (P< 0.001) in comparison with Manchega, which is explained by the differences in genotype and feeding behavior. These values are high and representative of the mid-lactation stage for each breed (Caja and Bocquier, 2000). Moreover, the SE values of milk yield were low as a result of the balanced groups.

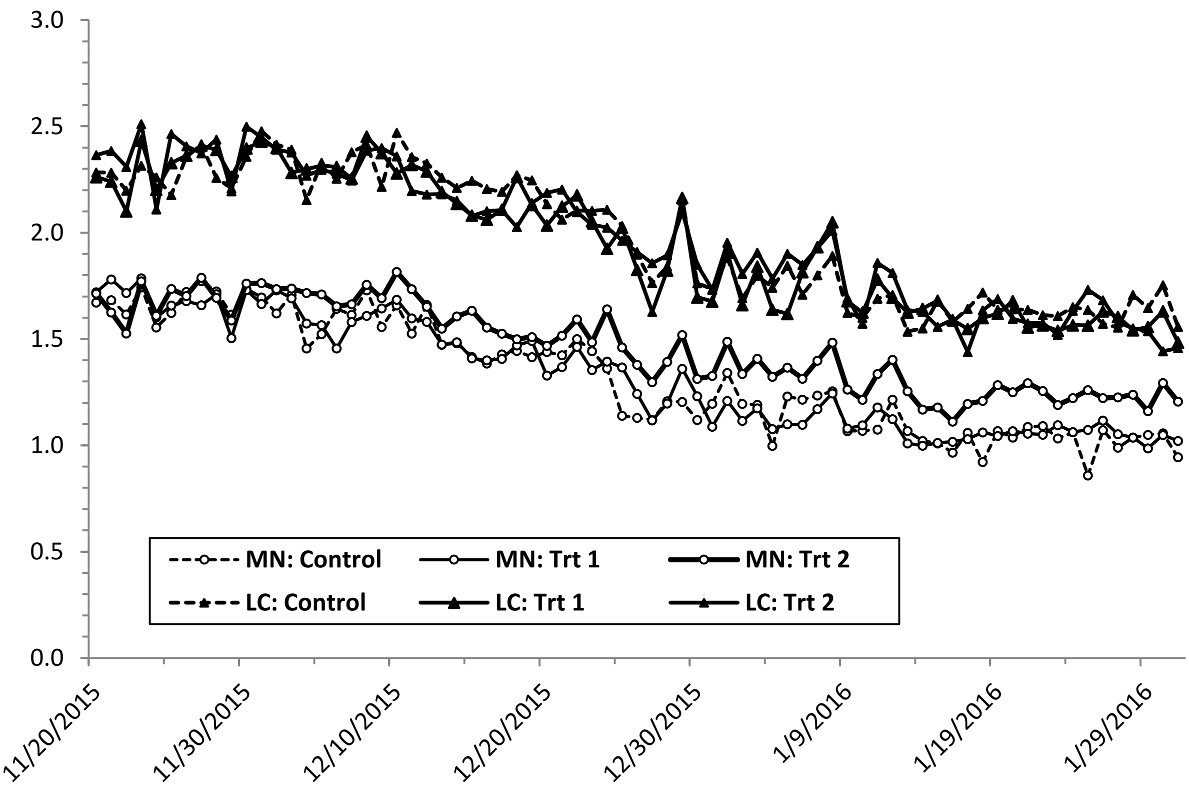
Table 6. Milk yield averages for each treatment at the sampling days of the experiment.

	Treatment[1]				Effect (*P* value)		
Yield (L/d)	CO	T1	T2	Mean± SE	CO vs.T1	CO vs.T2	T1 vs. T 2
Manchega							
d 60	1.67	1.68	1.68	1.68 ± 0.12	0.96	0.65	0.67
d 90	1.43	1.41	1.54	1.46 ± 0.11	0.96	0.65	0.67
d 120	1.02	1.07	1.24	1.11 ± 0.11	0.96	0.65	0.67
Lacaune							
d 60	2.28	2.30	2.36	2.31 ± 0.27	0.93	0.99	0.93
d 90	2.17	2.10	2.10	2.12 ± 0.29	0.93	0.99	0.93
d 120	1.63	1.59	1.62	1.61 ± 0.24	0.93	0.99	0.93

[1]Forage:concentrate ratio on DM basis: CO = Control (30:70), T1 = Medium (45:55) and T2 = High (60:40).

Moreover, for all treatments, the highest daily milk-yields were observed at approximately the d 60 of lactation, and thereafter those values were gradually falling down around 13% at d 90, and 24% at d 120, in Manchega ewes, and about 8% at d 90, and 24% at d 120 in Lacaune. The speed of decreasing the average milk yield was faster towards the end of lactation. It should be stressed that persistency might be dependent on the breed genotype (Ramırez Andrade *et al.* 2008), the Lacaune breed maintaining the persistency of milk production longer than the Manchega breed from d 60 to d 90, although both breeds were similar at the end of lactation. On the other hand, there was a special trend in the case of T2 (High) for Manchega breed were it was better preventing de decrease of milk yield. But in a general view, we can confirm that there are no clear differences between experimental treatments for both Manchega (P > 0.65 to 0.96) and Lacaune (P >0.93 to 0.99).

Figure 2. Average daily milk yield for each dietary treatment according to the forage:concentrate ratio (CO = Control, 30:70; T1 = Medium, 45:55; and T2 = High, 60:40) during the experiment.



The obtained results may indicate that the control diet was equilibrated and satisfactory to get the optimized amounts of daily milk production, according to the milk potential of each breed because there was no difference in milk yield by increasing level of concentrate between 3 treatments. So, higher nutritional stage was not a metabolic advantage for the ewes. Moreover, the *ad libitum* total mixed ratio used allowed them to adjust their intake to their requirements without inducing metabolic diseases.

3.2. Milk composition

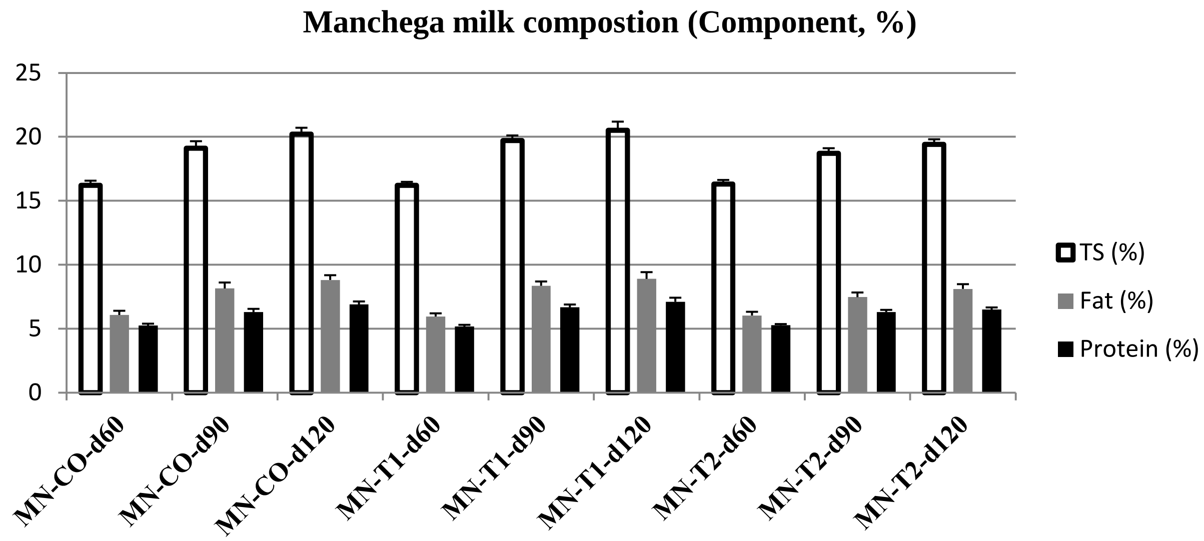
Unexpectedly, there was a fact that there were no marked effects on milk composition to the gradual increase of concentrate for the dietary treatments in both breeds, as it has been shown in Table 7 and Figure 3.

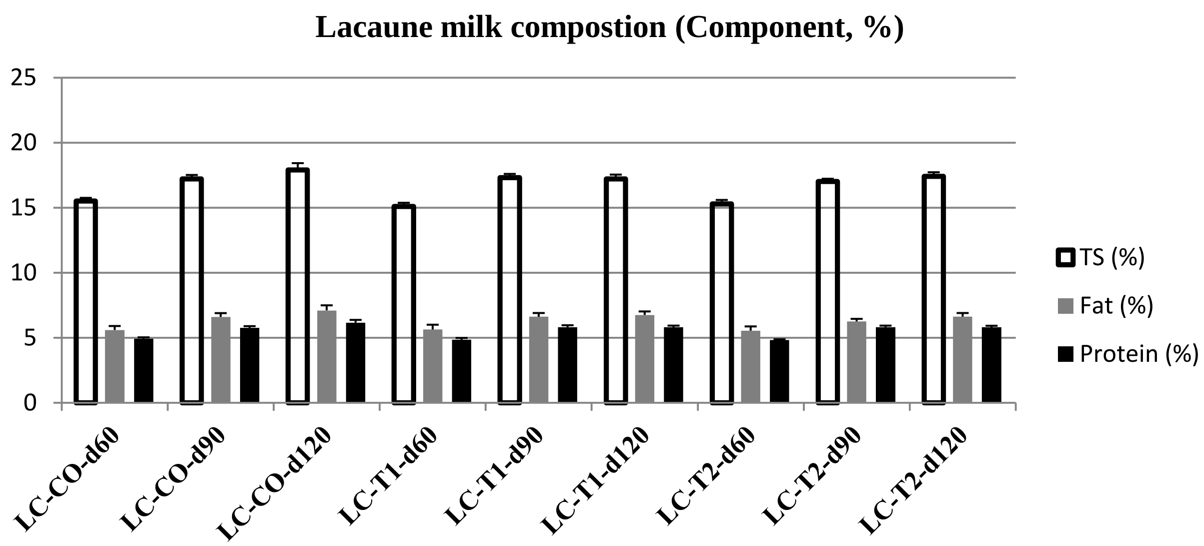
Table 7. Milk composition by ewe breed and stage of lactation for each dietary treatment during the experiment.

	Treatment[1]				Effect (P value)		
Item	CO	T1	T2	Mean± SE	CO vs.T1	CO vs.T2	T1 vs. T 2
Manchega							
Solids (%)							
d 60	16.2	16.2	16.3	16.2 ± 0.4	0.89	0.80	0.70
d 90	19.1	19.7	18.7	19.2 ± 0.6	0.89	0.80	0.70
d 120	20.2	20.5	19.4	20.0± 0.7	0.89	0.80	0.70
Fat (%)							
d 60	6.07	5.95	6.03	6.02± 0.33	0.96	0.67	0.65
d 90	8.14	8.35	7.46	7.98± 0.47	0.96	0.67	0.65
d 120	8.81	8.89	8.11	8.60 ± 0.53	0.96	0.67	0.65
Protein (%)							
d 60	5.25	5.17	5.27	5.23 ± 0.29	0.83	0.85	0.70
d 90	6.29	6.66	6.30	6.42± 0.26	0.83	0.85	0.70
d 120	6.89	7.10	6.49	6.83 ± 0.32	0.83	0.85	0.70
ECM (L/d)							
d 60	1.47	1.45	1.47	1.46 ± 0.11	0.81	0.57	0.71
d 90	1.50	1.54	1.57	1.54 ± 0.09	0.81	0.57	0.71
d 120	1.15	1.24	1.33	1.24 ± 0.11	0.81	0.57	0.71
Lacaune							
Solids (%)							
d 60	15.5	15.1	15.3	15.3 ± 0.3	0.78	0.78	0.98
d 90	17.2	17.3	17.0	17.2 ± 0.3	0.78	0.78	0.98
d 120	17.9	17.2	17.4	17.5 ± 0.5	0.78	0.78	0.98
Fat (%)							
d 60	5.59	5.63	5.54	5.59 ± 0.37	0.87	0.62	0.70
d 90	6.60	6.61	6.25	6.49± 0.29	0.87	0.62	0.70
d 120	7.10	6.75	6.62	6.82 ± 0.39	0.87	0.62	0.70
Protein (%)							
d 60	4.92	4.84	4.83	4.86 ± 0.14	0.80	0.90	0.90
d 90	5.76	5.81	5.81	5.79 ± 0.15	0.80	0.90	0.90
d 120	6.16	5.80	6.00	5.99 ± 0.21	0.80	0.90	0.90
ECM (l/d)							
d 60	1.90	1.87	1.92	1.90 ± 0.20	0.74	0.77	0.95
d 90	2.03	1.96	1.92	1.97± 0.25	0.74	0.77	0.95
d 120	1.61	1.51	1.54	1.55 ± 0.23	0.74	0.77	0.95

[1]Forage:concentrate ratio on DM basis: CO = Control (30:70), T1 = Medium (45:55) and T2 = High (60:40).

Figure 3. Milk composition profile at sampling days of Manchega (MN) and Lacaune(LC) dairy ewes in total solid (TS), milk fat concentration (Fat) and milk protein concentration (Protein). According to the forage:concentrate ratio (CO = Control, 30:70; T1 = Medium, 45:55; and T2 = High, 60:40) of the diet.





The genotype (i.e., level of production) was no significant and not taken into account as a key factor of the response to nutrition in this case. Agreeing the lower milk yield recorded for the Manchega breed, their milk had more total milk solid content than the Lacaune ($P< 0.05$). Theoretically, it is proven that the total solid and milk contents are negatively correlated. Reasonably, there are genetic and behavior (i.e., eating and drinking) features leading the

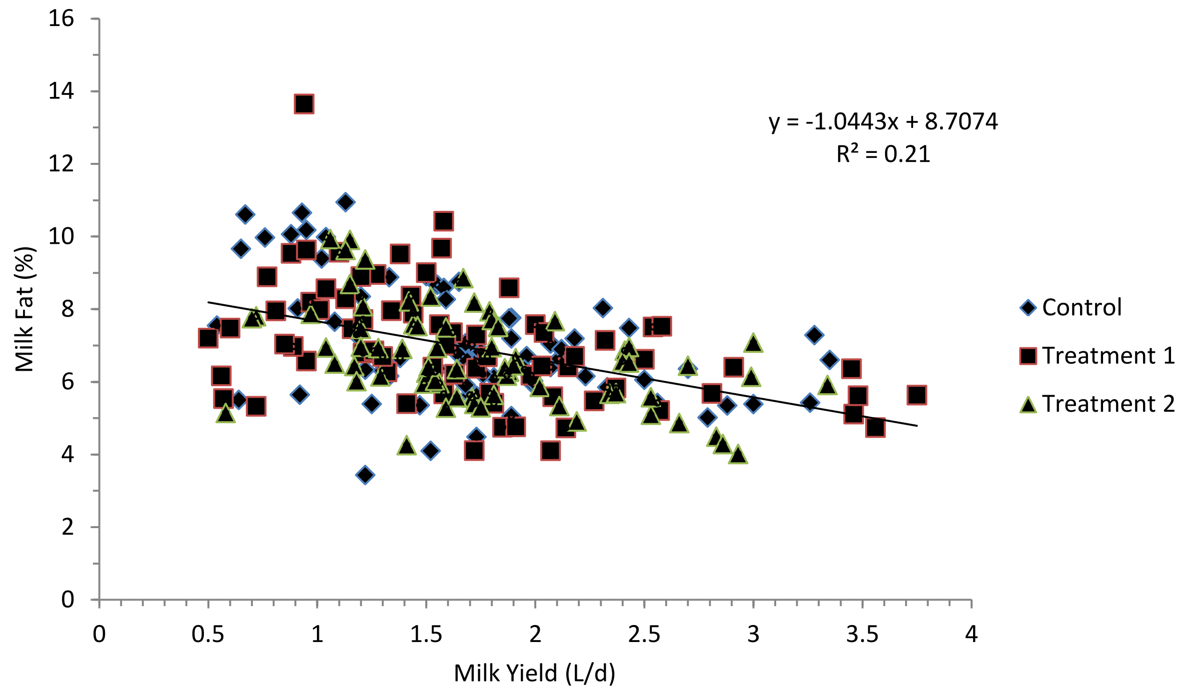
Lacaune to produce a greater milk yield that cannot sustain high total milk solids content like in the Manchega.

Consequently, it was not unexpected that, as lactation advanced, with the above discussed trend to reduce milk yield, the total milk solid content of all 3 treatment ewes increased from d 60 to 120 in both breeds ($P < 0.05$) with commonly recorded in several studies (Pavic *et al*. 2002, Kuchtik *et al.* 2008). Compositional changes with the stage of lactation were more marked in the Manchega than in the Lacaune, as recognized for these breeds (Ramırez Andrade *et al.* 2008). Hopefully, it seemed that the high nutrient intake was effective to keep the milk quality high at the end of lactation.

The main studied components of milk in this experiment were fat and protein because their main contribution to cheese production. According to the balanced ewe groups, they have similar mean values of fat and protein by breed in the start of experiment. As expected we also observed an increase in fat and protein concentrations from the start to the end of the experiment throughout lactation. So the positive correlations of fat and protein contents to the total solid were confirmed (data not shown). One more time, despite of the general tendency of increasing value in fat and protein concentrations, still there were different changing rates of those values by breed. There was a greater increase in fat content of Manchega ewes (approximately 30%: from 6.02% at d 60 to 8.60% at d 120) than in Lacaune ewes (approximately 18%: from 5.59% at d 60 to 6.82% at d 120). No similar phenomenon was found in protein.

Although, slight differences were observed between breeds in responding to the experimental treatments, there were no significant changes of fat and protein contents by effect of the dietary treatments applied. As a result, the ECM values of the experimental treatments were similar because of the close values in milk composition. It is also remarkable that this energy corrected milk was in most cases lower than the actual milk yield values achieved and its values were quite constant during de first experimental period (d 30 to 60). This lactation trend, showing a plateau is indicating a high persistency for all the dietary treatments used in both breeds as a result of a positive energy balance. Moreover, the feeding conditions used seem to be fully adequate for dairy sheep, despite their breed and level of milk production. As expected and shown in Figures 4a and 4b, milk yield was negatively correlated to milk fat and protein contents, although the correlation was positive when daily yields of milk components were considered.

Figure 4a. Correlations of milk fat content and fat yield with the daily milk yield of the experimental dairy ewes.



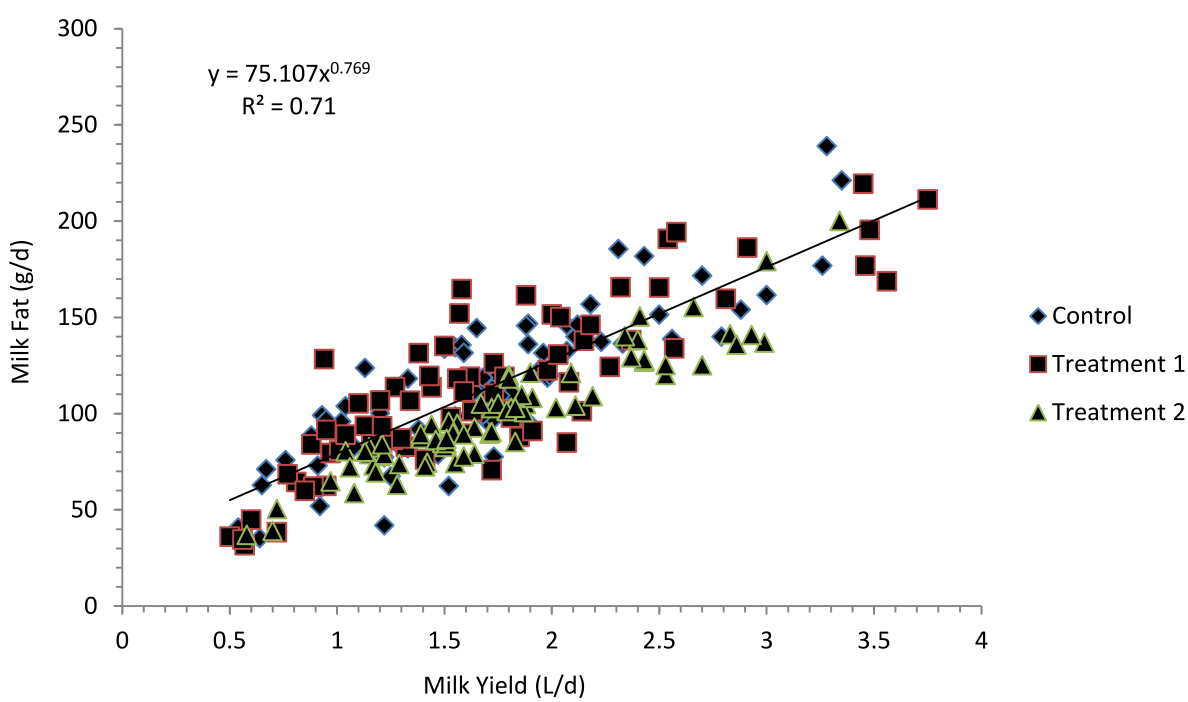
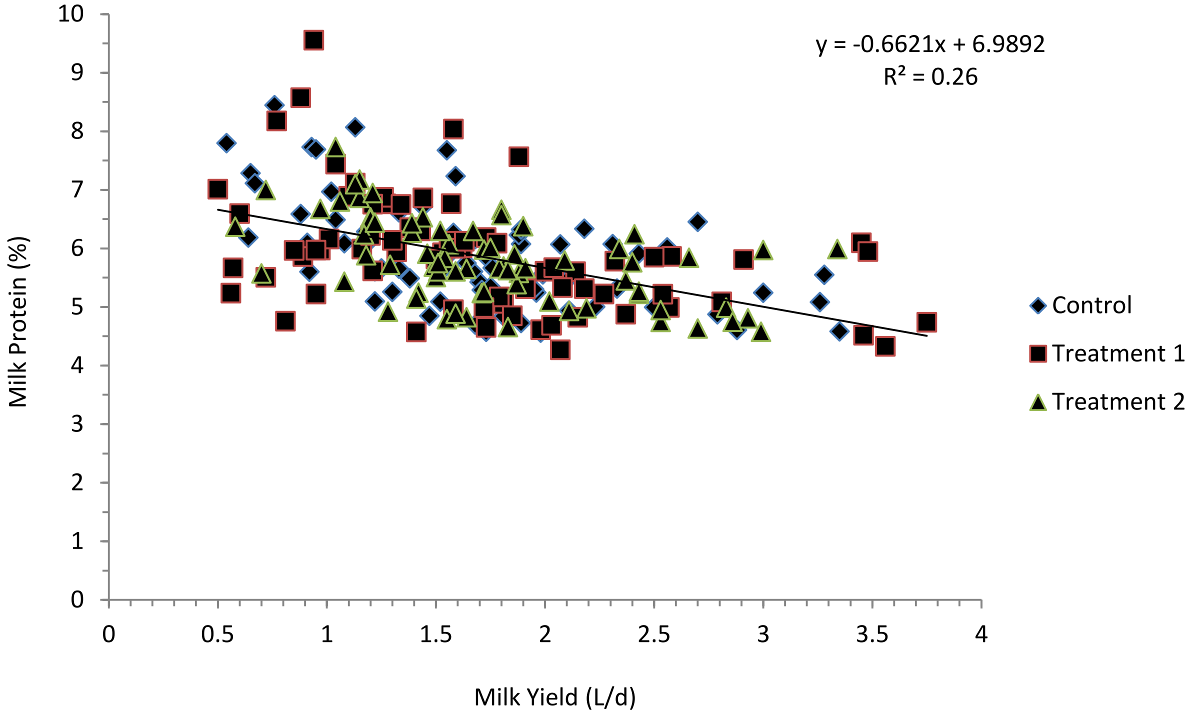
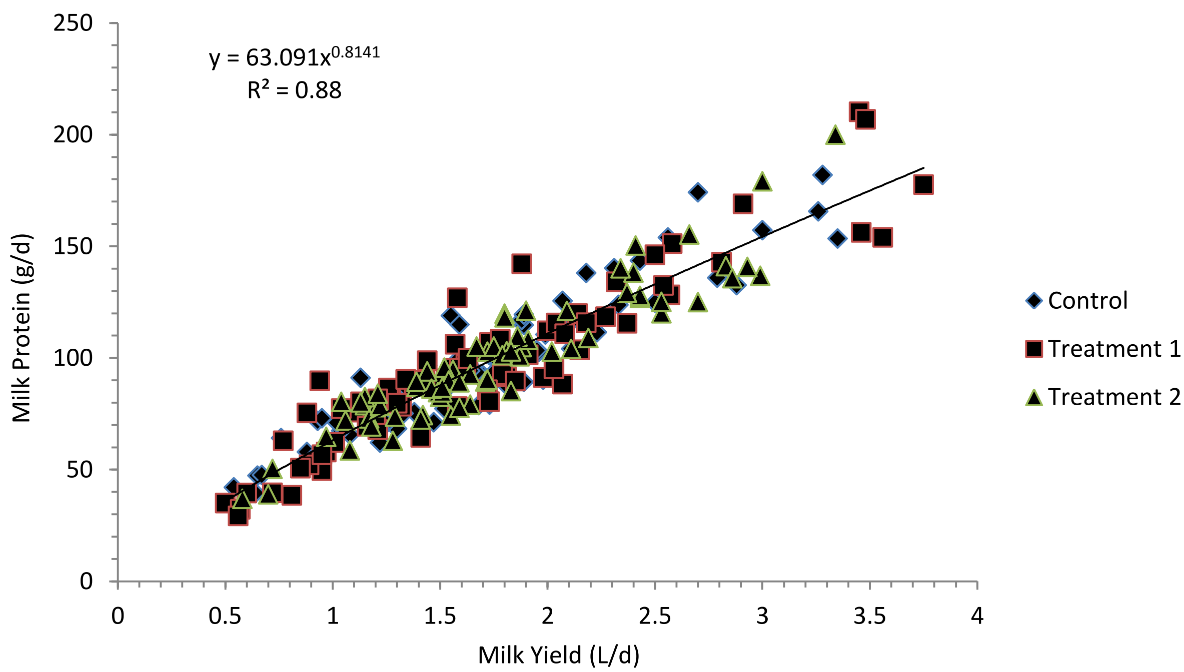


Figure 4b. Correlations of milk protein content and protein yield with the daily milk yield of the experimental dairy ewes.





Interestingly, there were strong similarities of the correlations obtained in our experiment with data from Manchega and Lacaune ewes and those of the previously reported paper from Sarda ewes (Pulina *et al.* 2006), despite of the greater amount of individual data of this paper. Hence, as we can confirm that the breed did not play a key role in the relationship between yield and composition in both studies. Moreover, when values were discriminated by feeding treatments, we can easily observe that they were distributed in the whole range of variation of milk yield values. So, we confirmed that the experimental treatments did not make any important effect able to separate the milk composition between them.

3.3. Cheese-making performance

There was an improvement of the average rennet coagulation time (RCT) of the milk of both breeds from the start to the end of the experiment, with no significant effects of the dietary treatments, as shown in Table 8 and Figure 5.

The reduction of RCT values, leading to a faster milk coagulation as lactation advanced, was more marked in the Manchega (from 16.9 min at d 90 to 11.9 min at d 120) than in the Lacaune (from 15.1 min at d 90 to 13.2 min at d 120). Similar reduction was detected in dairy cows (Jõudu *et al.* 2008, Guinee *et al.* 1997), despite the difference between species also leading to different range of values. This reduction can be partly explained by the increase of protein concentration throughout lactation and because the high contribution of protein to build up the curd structure. Nevertheless, despite of the similarly starting point values, there was a more marked decline of RCT in Manchega milk (about 29.5%) in comparison with Lacaune (around 12.5%). This may be a result of the different expression of breed genotype to the lactation stage and feeding strategy. As we have already seen in Table 7, there were marked increases of total solid and milk composition as lactation advanced, especially with regard to fat and protein contents. These changes make the milk easier and faster to coagulate as the protein concentration increased. Values of SE were proportionally higher than those of Table 6 and 7, because higher intrinsic variability of these Analyses.

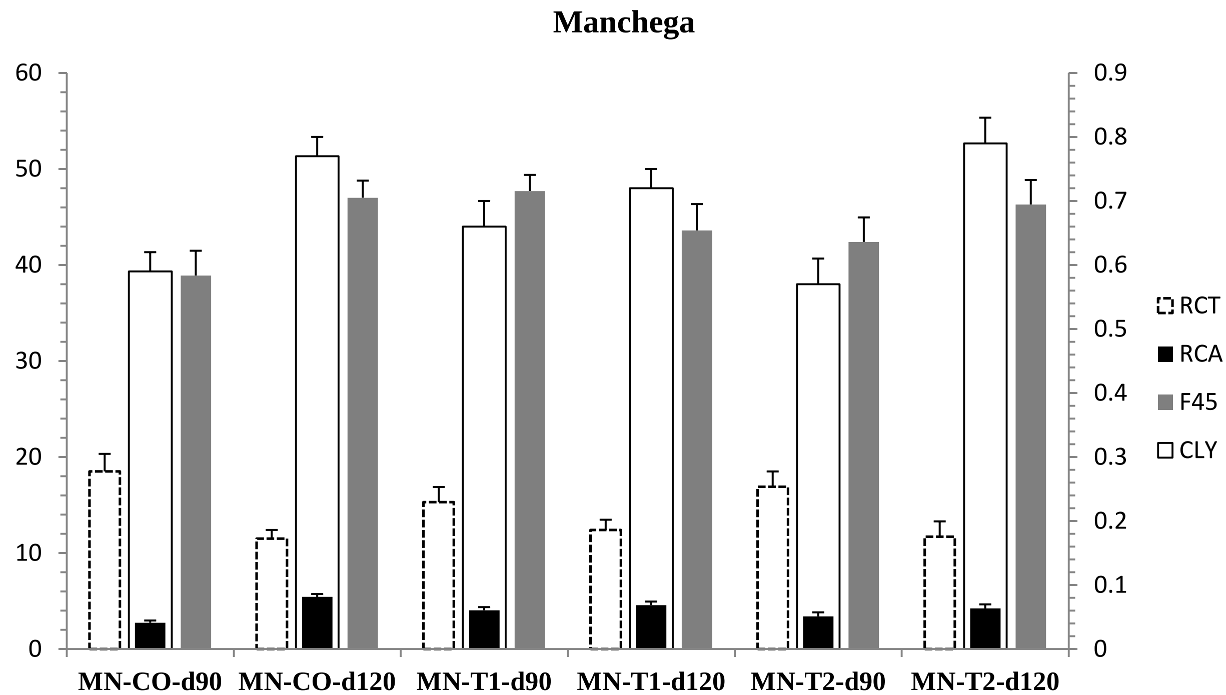
With regard to firmness at min 45 (F45), values of Manchega (43.0 at d 90 and 45.6 at d 120) were on average greater than those of Lacaune (34.1 at d 90 and 37.7 at d 120). Moreover, values of F45 slightly increased to the end of lactation, although the differences were no significant. Milk of Manchega showed a greater firmness than that from Lacaune throughout lactation. Most cheese-yielding performances of each breed during the lactation period studied were different despite using the same diet as a consequence of their different milk composition, being LCY on fresh weight basis and on average 0.69 ± 0.04 and 0.59 ± 0.03 mg/mL in Manchega and Lacaune ewes (*P*< 0.05), respectively.

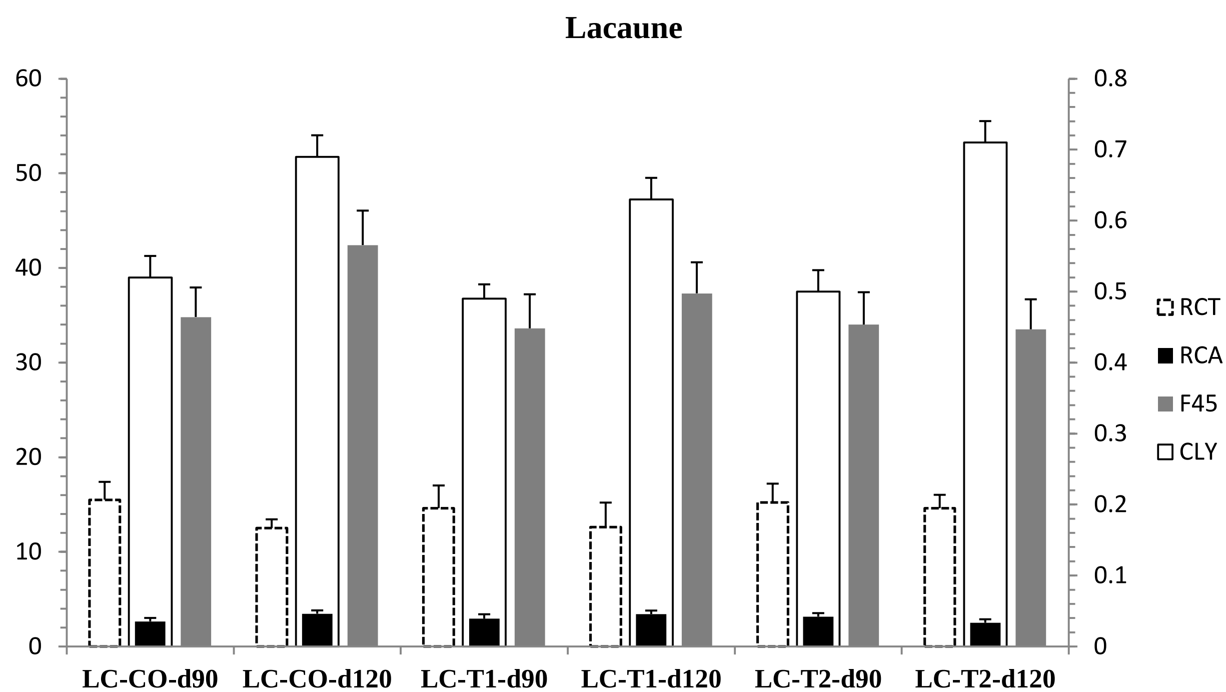
Table 8. Cheese yielding traits of the milk of dairy ewes according to dairy breed and the forage:concentrate ratio (CO = Control, 30:70; T1 = Medium, 45:55; and T2 = High, 60:40) at the different sampling days of experiment.

	Treatment				Effect (*P* value)		
Item	CO	T1	T2	Mean± SE	CO vs.T1	CO vs.T2	T1 vs. T 2
Manchega							
RCT[1]							
d 90	18.5	15.3	16.9	16.9 ± 1.8	0.80	0.89	0.90
d 120	11.5	12.4	11.7	11.9 ± 1.6	0.80	0.89	0.90
RCA[2]							
d 90	2.74	4.04	3.40	3.40 ± 0.43	0.89	0.87	0.42
d 120	5.42	4.57	4.23	4.74 ± 0.43	0.89	0.87	0.42
F45[3]							
d 90	38.9	47.7	42.4	43.0 ± 2.7	0.60	0.78	0.68
d 120	47.0	43.6	46.3	45.6 ± 2.7	0.60	0.78	0.68
LCY[4]							
d 90	0.59	0.66	0.57	0.61 ± 0.04	0.92	1.00	0.94
d 120	0.77	0.72	0.79	0.76 ± 0.04	0.92	1.00	0.94
Lacaune							
RCT[1]							
d 90	15.5	14.6	15.2	15.1 ± 2.4	0.85	0.60	0.33
d 120	12.5	12.6	14.6	13.2 ± 2.6	0.85	0.60	0.33
RCA[2]							
d 90	2.64	2.93	3.14	2.90 ± 0.46	0.82	0.70	0.47
d 120	3.45	3.41	2.49	3.12 ± 0.37	0.82	0.70	0.47
F45[3]							
d 90	34.8	33.6	34.0	34.1 ± 3.6	0.54	0.33	0.45
d 120	42.4	37.3	33.5	37.7 ± 3.6	0.54	0.33	0.45
LCY[4]							
d 90	0.52	0.49	0.50	0.50 ± 0.03	0.72	1.00	0.76
d 120	0.69	0.63	0.71	0.68 ± 0.03	0.72	1.00	0.76

[1]Rennet coagulation time (min); [2]Rate of curd aggregation; [3]Firmness at 45 min; [4]Laboratory cheese yield (g/mL).

Figure 5. Cheese-making performance traits (RCT, Rennet coagulation time; RCA, Rate of curd aggregation; F45, firmness at 45 min; LCY, Laboratory cheese yield) of Manchega and Lacaune dairy ewes according to the forage:concentrate dietary treatments (Control, CO; Medium, T1; High, T2).





Despite of the changes in milk total solids with the stage of lactation in both breeds we did not detect effects on RCT values for the 3 experimental feeding treatments. Moreover, RCA values were also similar between dietary treatments. It is exciting that there was an improvement of milk's cheese-yielding until the end of lactation (d 90 vs. 120) being the increase greater ($P < 0.05$) in Lacaune (26.5%) than in Manchega (19.7%).

These results may be explained by the fact that ewes received and equilibrated diet and they adapted the intake of nutrients to produce a moderate increase of body reserves. Under the feeding conditions used in our experiment the forage:concentrate ratio did not show detectable effects on milk composition and cheese-yielding features of ewe's milk.

CONCLUSIONS

The results of the present study show that increasing the concentrate proportion in the diet of dairy ewes, under *ad libitum* total mixed ration conditions and using a good forage, did not produce significant changes in the lactation performances (milk yield, milk composition) and cheese-yielding features of their milk (rennet coagulation and cheese-yielding traits) of both Manchega (medium milk yielding, rich milk composition) and Lacaune (high milk yielding, medium milk composition) ewes. Although, breed traits lead to many differences in lactation performances through lactation these differences were not significantly affected by the dietary treatments studied.

In the economy meaning of this experimental project, it was proven that the control diet, consisting of 30% of concentrate and 70% forage (regular alfalfa hay), may be considered as enough for mid- and high-yielding dairy sheep during the entire lactation. So, in most conditions, a higher quantity of concentrate will lead to an undesirable ruminant function and higher feeding costs without the expectation of getting more profit from milk and cheese-making production.

In conclusion and under the medium-term conditions of this experiment, the optimal diet to be used in dairy ewes should depend on the forages and concentrate market prices and not on dairy performances.

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